Detection of embryo mortality and hatch using thermal differences among incubated chicken eggs[1]

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[1] Please cite as:

Q. Tong, C. Romanini, V. Exadaktylos, I. McGonnell, D. Berckmans, C. Bahr, H. Bergoug, N. Roulston, M. Guinebretiere, N. Eterradossi, R. Verhelst and T. Demmers. Detection of embryo mortality and hatch using thermal differences among incubated chicken eggs. *Livestock Science*, 183: 19-23, 2016.

Abstract

Accurate diagnosis of both the stage of embryonic mortality and the hatch process in incubated eggs is a fundamental component in troubleshooting and hatchery management. However, traditional methods disturb incubation, destroy egg samples, risk contamination, are time and labour-intensive and require specialist knowledge and training. Therefore, a new method to accurately detect embryonic mortality and hatching time would be of significant interest for the poultry industry if it could be done quickly, cheaply and be fully integrated into the process. In this study we have continuously measured individual eggshell temperatures and the corresponding micro-environmental air temperatures throughout the 21 days of incubation using standard low-cost temperature sensors. Moreover, we have quantified the thermal interaction between eggs and air by calculating thermal profile changes (temperature drop time, drop length and drop magnitude) that allowed us to detect four categories of egg status (infertile/early death, middle death, late death and hatch) during incubation. A decision tree induction classification model accurately (93.3%) predicted the status of 105 sampled eggs in comparison to the classical hatch residue breakout analyses. With this study we have provided a major contribution to the optimization of incubation processes by introducing an alternative method for the currently practiced hatch residue breakout analyses.

Keywords: egg breakout, eggshell temperature, embryo status, hatching time.

Introduction

Hatchability is key for assessing incubation results. Thus, investigating hatching failures is an increasingly recognized concern for the modern poultry industry in attempting to uncover the basis of egg fertility and embryonic mortality (Sellier et al., 2006). Besides, Non-viable eggs also take up space that can be used for fertile eggs and a potential source of bacteria and/or fungi and

43 can thus cause contamination. During incubation, egg candling is normally carried out in the
44 middle of the process (day 10) or during transfer (day 18), in order to identify infertile eggs and
45 mortality. However, egg breakout analyses requires invasive intervention, destroys egg samples,
46 kills embryos, risks contamination, is time and labour-intensive and requires specialist
47 knowledge and training (Sellier et al., 2006, Liu and Ngadi, 2013). Detection of infertility and
48 mortality is not the only issue, investigation of the hatch evolution is also very important to
49 evaluate uniformity of the batch. In practice, the moment of hatch is examined by taking several
50 hatchery baskets out of the incubator and checking the number of chicks hatched. However, this
51 procedure may require the door of the incubator to be opened and is carried out several times
52 during the process which may significantly affect the incubation conditions and interrupt the
53 hatching process (Tong et al., 2015). Therefore, there is an increased interest in an alternative
54 and less invasive method for the detection of egg fertility and monitoring of embryo mortality
55 and hatch. This paper attempts to show the benefit of using eggshell temperature sensors during
56 the whole incubation to quantify thermal profile differences among infertile eggs, eggs
57 containing dead embryos at different developmental stages (early, middle and late) and eggs that
58 succeed in hatching.

59 **Material and methods**

60 Ross 308 eggs (Henry Stewart & Co. Ltd., Lincolnshire, United Kingdom) were incubated and
61 hatched in a custom small-scale incubator (Petersime NV., Zulte, Belgium) using a standard 21-
62 day incubation program. Twenty out of six hundred eggs were randomly picked and individually
63 labelled as focal eggs in each incubation trial to serve as samples for the current study. In total,
64 120 focal eggs from six repetitions were analysed. Standard low-cost contact temperature sensors
65 (Romanini et al., 2013) were attached to the equator of the focal egg eggshells. Another

66 temperature sensor was positioned 1 cm away from each focal egg to record the corresponding
67 micro-environmental air temperature (T_{air}). The eggshell temperatures (T_{egg}) of each focal egg
68 and T_{air} were recorded every minute throughout the entire incubation.
69 At the end of incubation, hatch residues were evaluated by an expert in breakout analysis
70 (Petersime NV, Zulte, Bekgium). Egg status was determined according to the developmental
71 stage of the dead embryo (Hamburger and Hamilton, 1992) and allocated into the following
72 categories: infertile (INF), early death (ED), middle death (MD) and late death (LD). Cracked
73 and contaminated eggs were excluded from the analyses. This study had an ethical approval from
74 the Royal Veterinary College Ethics and Welfare Committee.
75 Both T_{egg} and T_{air} time series data were processed in Matlab® (The Math Works, Inc., Natick,
76 United States) using built-in codes and functions of the Captain Toolbox (Taylor et al., 2007).
77 The temperature difference (ΔT) between T_{egg} and T_{air} was calculated and filtered to produce the
78 $\Delta T_{filtered}$ signal representing the final thermal profile. The $\Delta T_{filtered}$ signal was further processed
79 using a 10-hours average window approach to investigate differences in temperature. The
80 following parameters of an identified temperature drop in $\Delta T_{filtered}$ were quantified for the MD,
81 LD and H focal eggs (Figure 1): 1) the time when the lowest drop occurred (drop time); 2) the
82 duration of the drop from a maximum local value to the minimum local value (drop length); and
83 3) the temperature scale of the drop (drop magnitude).
84 **Insert Figure 1**
85 Statistics were performed using the statistical software package Minitab® (Minitab Inc., State
86 College, United States). Initially, the thermal profiles of focal eggs were grouped into one
87 category of egg status (INF, ED, MD, LD or H) according to the results of the hatch residue
88 breakout analyses. The Anderson-Darling (Anderson and Darling, 1954) and the Bartlett's tests

(Ridgman, 1990) were used to test normality and homogeneity of variances, respectively. The parameters extracted from $\Delta T_{filtered}$ were summarized using descriptive statistics (mean followed by the standard error of the mean). A single ANOVA followed by post hoc test, with a significance level of 0.05, was used to test the differences in drop length and drop magnitude among the egg categories.

The data set was classified using a decision tree induction model (Quinlan, 1986). Thermal profile derived parameters (temperature drop time, drop length and drop magnitude) were inputs towards the classification of the egg status (INF, ED, MD, LD or H) as output. The application WEKA 3.6.9 (University of Waikato, New Zealand) was used to develop a J48 decision-based classifier (Hall et al., 2009) with a 10-fold cross validation approach. Egg status classified by the decision tree model, was compared to the reference status from breakout analyses. The classification performance was expressed in terms of binary classification statistics (Olson and Delen, 2008): TP rate (rate of true positives); FP rate (rate of false positives); and ROC-curve (the ability of performing correctly classification).

Results

The 105 focal eggs were grouped according to the results of the hatch residue breakout analysis, as following: INF (n=15), ED (n=3), MD (n=12), LD (n=11) and H (n=64). The thermal profile interactions between T_{egg} and T_{air}, throughout the entire incubation time (512 hours), of two focal eggs from each egg status category are illustrated, as examples, in Figure 2.

Insert Figure 2

A common feature (notable temperature drops on Figures 2C, 2D and 2E) was found for all focal eggs categorized into MD, LD and H. Eggs in the status category INF/ED did not show the same pattern (Figure 2A and 2B). Those temperature drops were associated to the time of embryonic

mortality in the cases of MD and LD, or to the time which chicks emerge from their shells in the case of H eggs. Figure 3 shows the normal distribution curves of the temperature drop time for the MD, LD and H eggs. This result shows overlap between the temperature drop time registered for the egg categories MD and LD or, most clearly for LD and H.

Insert Figure 3

In addition, quantitative differences were identified in the thermal profiles (temperature drop length and drop magnitude) of MD, LD and H eggs (Table 1). The temperature drop length found in the H eggs (mean of 6.78 hours) was significantly lower than LD (15.82 h) and MD (18.75h) ($P<0.05$). Furthermore, differences in drop magnitude were found among the egg status categories. The highest temperature drop was obtained for the H eggs with a mean value of 0.73 °C ($P<0.01$). MD and LD eggs showed smaller averaged drop magnitudes of 0.19 °C and 0.30 °C, respectively.

Insert Table 1

Figure 4 shows the results of a binary decision tree and the thresholds used for classification into one of the four outcome egg status (INF/ED, MD, LD and H) according to the thermal profiles of the interaction between T_{egg} and T_{air}. At the top level of the decision tree there is the root node, at which the classification begins. It tests all focal eggs for temperature drop time <= 455 h of incubation. Instances that satisfy this condition are passed down to the left of the tree to the second root node. It corresponds to a new test (temperature drop time <= 0 h), meaning that there is no such notable temperature drop on the thermal profile ($\Delta T_{filtered}$). If this is the case, this test passed once more down to the left reaching a leaf node INF/ED and no more tests are needed. Focal eggs are classified as MD if the temperature drop time <= 383 h, or as LD if the temperature drop time is > 383 h but <= 455 h of incubation. From the top root node to the right,

135 focal eggs with a temperature drop time > 455 h are further tested at intermediate nodes
136 (temperature drop length and drop magnitude) to be classified into one single egg status category
137 (LD or H).

138 **Insert Figure 4**

139 The performance of the decision tree classification model is shown in Table 2. The overall
140 success rate of the classification model was 93%. Each element in Table 2 is a count of focal
141 eggs. Rows represent the reference egg status categories classified by hatch residue breakout
142 analyses and columns represent the predicted egg status by the decision tree model. A total of 98
143 out of 105 focal eggs were correctly classified. The model was 100% precise at classifying
144 INF/ED eggs. 10 out of 12 MD eggs were correctly classified and 2 were incorrectly categorized
145 as LD. The highest error rate was in the LD eggs with 3 errors in 11 focal eggs due to the limited
146 sample size. The classification model correctly classified 62 out of 64 H eggs. Detailed accuracy
147 results for each status category are shown in Table 3. A ROC-curve value of 1 represents a
148 perfect classifier while values approaching 0.5 indicate a classifier with reduced ability,
149 comparable to random guessing. ROC-curve values were higher than 0.9 for each egg status
150 category.

151 **Insert Table 2**

152 **Insert Table 3**

153 **Discussion**

154 The quantification of the changes in the thermal profile interaction between T_{egg} and T_{air} for the
155 whole incubation period allowed us to identify four categories of egg (fertility/early death,
156 middle death, late death and hatch). As expected, the decision tree classification model indicated
157 'drop time' as the criteria holding the greatest information. We cannot distinguish between an

infertile egg and an egg with early embryonic mortality because they showed similar thermal profiles throughout incubation. Furthermore, drop time alone seems unlikely to be enough to distinguish classes of embryo status in the overlapping regions when our results are extrapolated to a population level. Temperature drops were identified when embryos died or hatch as a response to an abrupt change in embryonic heat production. This allowed us to precisely determine the time for embryonic mortality and hatch. The quickest and largest temperature drop was observed for hatched eggs. After external pipping, the chick emerges from the egg and causes huge amount of heat release due to evaporation of the fluid left in the internal eggshell membrane by the recently hatched chick (Romanini, et al., 2015). Therefore, the temperature drop caused by hatch was different from the temperature drop caused by embryo death in terms of drop length and drop magnitude. We have developed a classification model with a small number of misclassification (7 errors out of 105 focal eggs), corresponding to an overall 93.3% accuracy. With the increase of egg samples, especially for late dead eggs, the classification model could be fine-tuned and yield even higher accuracy.

Conclusion

A method was proposed in this study to determine the egg status using the temperature sensors based on the different thermal profile changes throughout the incubation period. Accurate detection of embryo status and the moment of hatch in commercial hatcheries would be of significant interest to the poultry industry if it can be done quickly and cheaply. The results presented herein correspond to the absolute first step, distinction of thermal profiles of eggs from different status, towards the development of future automated monitoring systems for incubated chicken eggs.

Acknowledgement

This study is part of the EU Marie-Curie Initial Training Network, BioBusiness project (FP7-PEOPLE-ITN-2008) and CNPq - Brazil, which the authors are grateful for the financial support.

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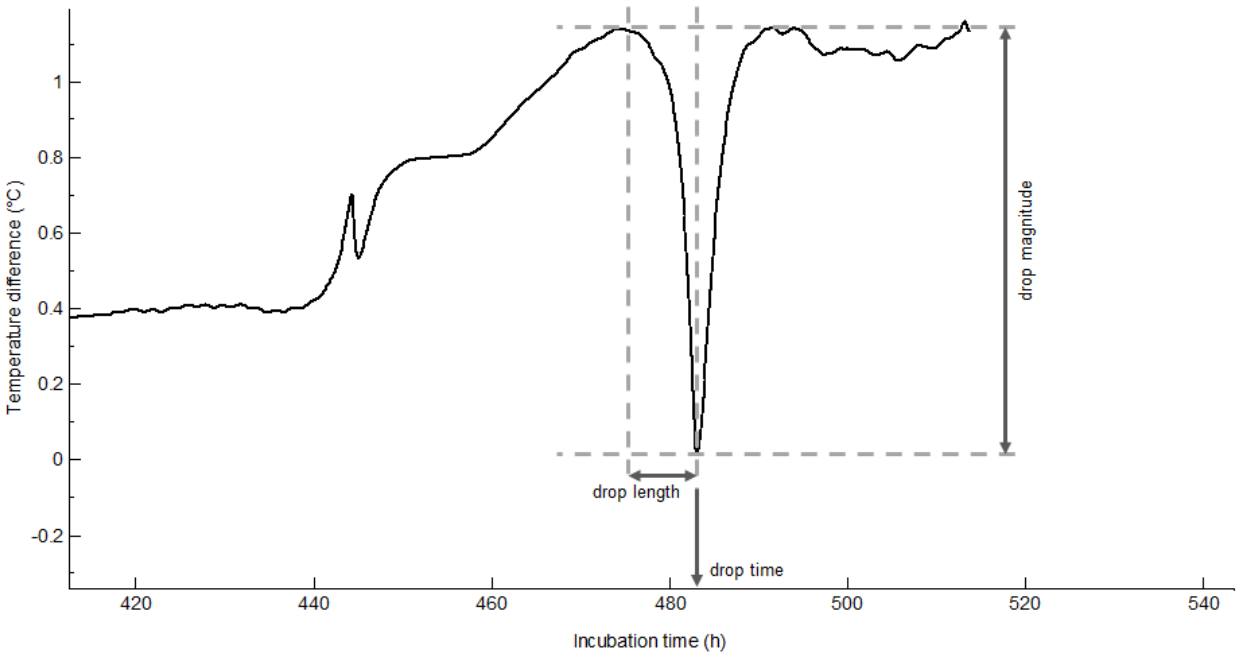
215 **Figure**



216

217 **Figure 1** An example of the quantitative characteristic (drop time, drop length and drop

218 magnitude) of the $\Delta T_{filtered}$ time series data.

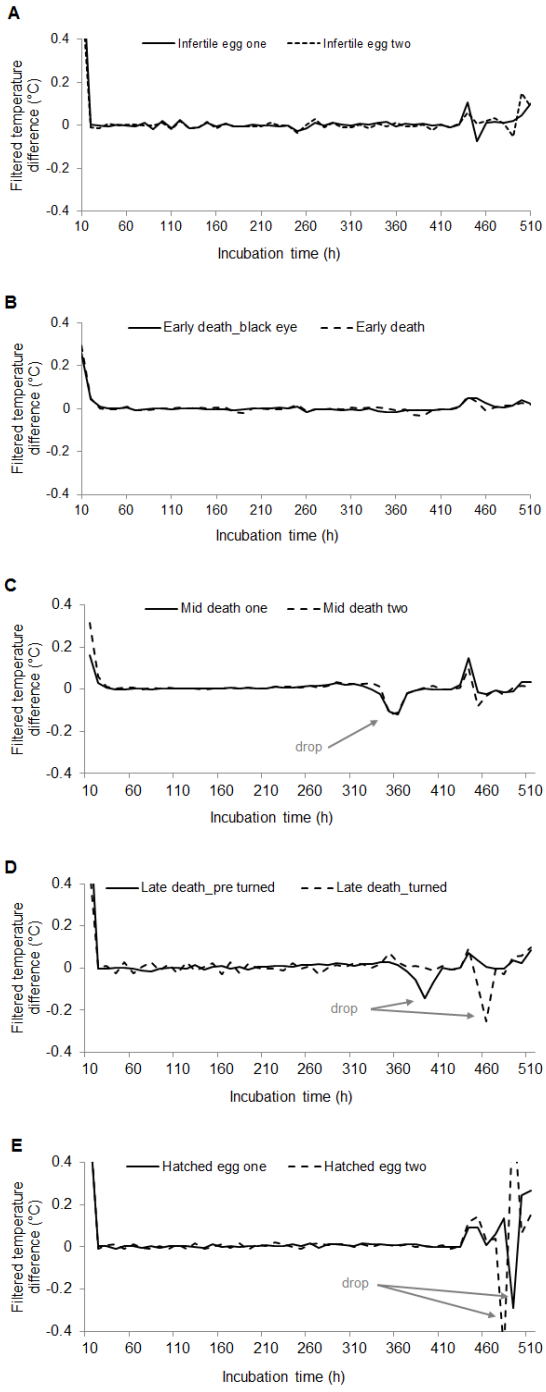
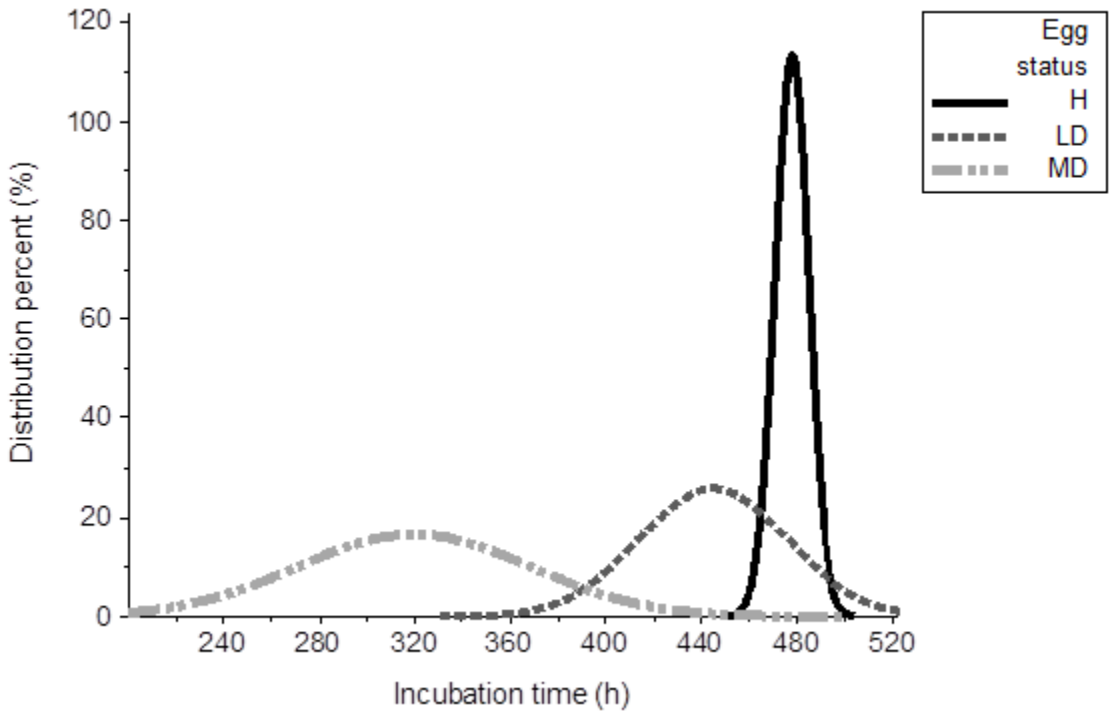


Figure 2 The corrected $\Delta T_{filtered}$ profiles throughout incubation of: A) Two early death eggs; B) Two mid death eggs; C) Two late death eggs; D) Two hatched eggs. The variation between 430-450 hours of incubation was caused by the temperature change during transfer of the eggs from incubation trays to hatching baskets.



224

225 **Figure 3** Fitted normal distribution curves for the time of temperature drop (drop time) occurred

226 in $\Delta T_{filtered}$ for MD, LD and H embryo status categories in the function of incubation time. MD =

227 middle death (n = 12), LD = late death (n = 11), H = hatched eggs (n = 64).

228

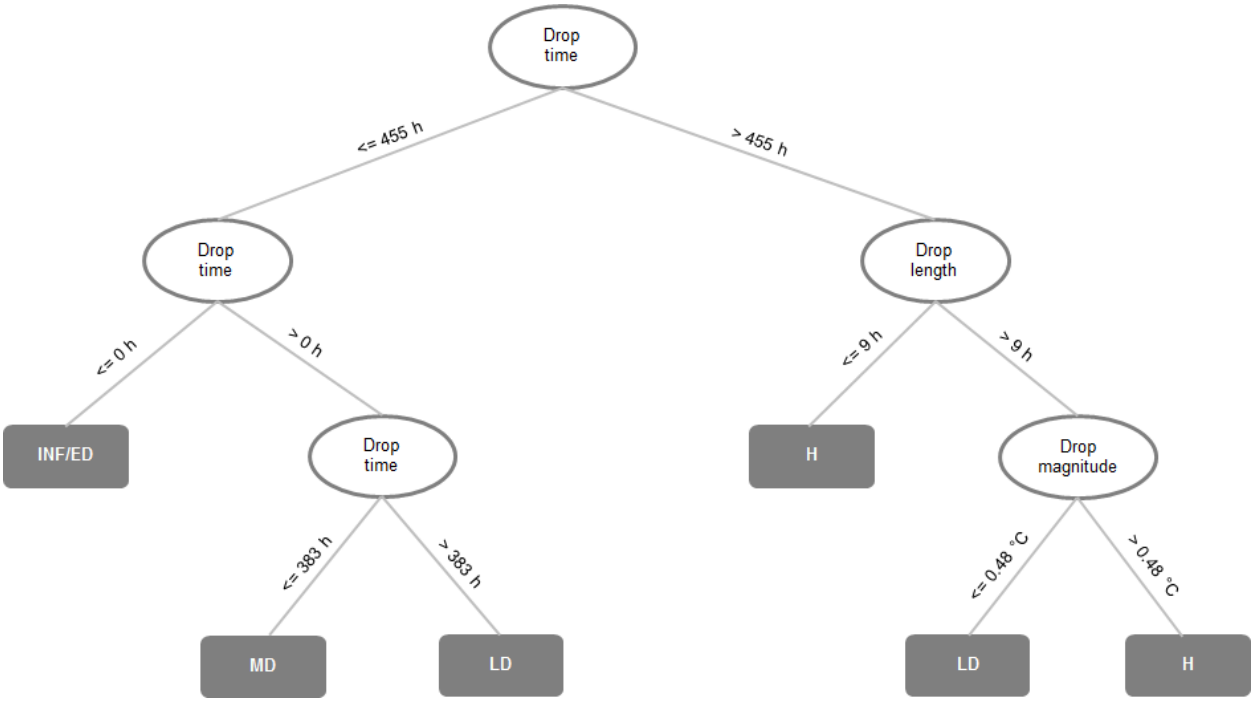


Figure 4 J48 decision tree classification model for egg status into the INF/ED, MD, LD and H categories with the specification of the classification rules thresholds. INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs.

Table Captions

Table 1 The comparisons of the drop length and drop magnitude among MD, LD and H focal eggs categories.

Item	Sample size	Drop length (h)	Drop magnitude (°C)
INF/ED	18	/	/
MD	12	18.75 ± 1.80^{a}	0.19 ± 0.02^{b}
LD	11	15.82 ± 1.90^{b}	0.30 ± 0.03^{b}
H	64	6.78 ± 0.14^{c}	0.73 ± 0.03^{a}

INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs; $^{a\text{-}c}$ means within the column with different superscripts differ significantly ($P<0.05$).

Table 2 Results of the classification tree in a confusion matrix form using a 10-fold cross-validation.

Reference status from hatch residue analyses	Classified by the decision tree model			
	INF/ED	MD	LD	H
INF/ED (n = 18)	18	0	0	0
MD (n = 12)	0	10	2	0
LD (n = 11)	0	1	8	2
H (n = 64)	0	0	2	62

INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs, n = sample size of focal eggs.

Table 3 Detailed accuracy results of the decision tree model by category of egg status

Egg status Category	INF/ED	MD	LD	H	Weighted Average
TP rate	1	0.83	0.73	0.97	0.933
FP rate	0	0.01	0.04	0.05	0.035
ROC-curve	1	0.99	0.91	0.99	0.985

INF/ED = infertile or early death, MD = middle death, LD = late death, H = hatched eggs, TP rate = rate of true positives, FP rate = rate of false positives, ROC-curve = classification ability.